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November 14, 2000

BOX PCT

Assistant Commissioner for Patents
Washington, D.C. 20231

PCT/JP99/02485
-filed May 13, 1999

Re: Application of Daikichi FUKUSHIMA, Shiro SHIBAYAMA and Hideaki TADA
NOVEL POLYPEPTIDES, cDNA ENCODING THE SAME, AND USE OF THEM
Our Ref: Q61459

Dear Sir:

The following documents and fees are submitted herewith in connection with the above application for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty:

- ☒ an executed Declaration and Power of Attorney.
- ☒ an English translation of the International Application and Declaration of the Translator.
- ☒ thirty-nine (39) pages of Sequence Listing.
- ☒ Notification Concerning Submission or Transmittal of Priority Document.
- ☒ International Preliminary Examination Report (foreign language).
- ☒ an executed Assignment and PTO 1595 form.
- ☒ International Search Report, Information Disclosure Statement, Form PTO-1449, and a complete copy of each reference.

It is assumed that copies of the International Application, and any Articles 19 and 34 amendments as required by § 371(c) will be supplied directly by the International Bureau, but if further copies are needed, the undersigned can easily provide them upon request.

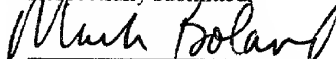
The Government filing fee is calculated as follows:

Total claims	<u>11</u>	-	20	=		x	\$18.00	=	<u>\$0.00</u>
Independent claims	<u>1</u>	-	3	=		x	\$80.00	=	<u>\$0.00</u>
Base Fee									<u>\$860.00</u>
Multiple Dependent Claim Fee									<u>\$270.00</u>
TOTAL FILING FEE									<u>\$1130.00</u>
Recordation of Assignment									<u>\$ 40.00</u>
TOTAL FEE									<u>\$1170.00</u>

Checks for the statutory filing fee of \$1130.00 and Assignment recordation fee of \$40.00 are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.492 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from May 14, 1998 based on Japanese Application No. P. Hei. 10-131815.

Respectfully submitted,



Mark Boland
Registration No. 32,197

MXB/amt

PATENT APPLICATION

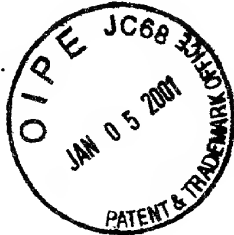
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Daikichi FUKUSHIMA, et al.

Appln. No.: 09/700,397

Filed: November 14, 2000



Group Art Unit: Not Yet Assigned

Examiner: Not Yet Assigned

For: NOVEL POLYPEPTIDES, cDNA ENCODING THE SAME, AND USE OF THEM

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 5, line 14, after "SEQ ID NOS." please delete "1" and insert --3--,

Page 5, line 15, please delete "6, 9 or 12" and insert --8, 11 or 14--,

Page 5, line 17, after "SEQ ID NOS." please delete "2, 5, 7, 10" and insert --1, 5, 6, 9--,

Page 5, line 18, please delete "13" and insert --12--,

Page 5, line 19, after "SEQ ID NOS." please delete "3, 8, 11" and insert --2, 7, 10--,

Page 5, line 20, please delete "14" and insert --13--,

Page 5, line 22, after "SEQ ID NOS." please delete "1" and insert --3--,

Page 5, line 23, please delete "6, 9 or 12" and insert --8, 11 or 14--,

Page 5, line 27, after "SEQ ID NOS." please delete "2, 5, 7, 10 or 13" and insert --1, 5, 6, 9 or 12--,

Page 5, line 29, after "SEQ ID NOS." please delete "2, 5, 7, 10, 13, 3, 8, 11 or 14" and insert --1, 5, 6, 9, 12, 2, 7, 10 or 13--,

Page 6, line 3, after "NOS." please delete "1, 4, 6, 9 or 12" and insert --3, 4, 8, 11 or 14--,

Page 6, line 5, after "SEQ ID NOS." please delete "1, 4, 6, 9" and insert --3, 4, 8, 11--,

PRELIMINARY AMENDMENT

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Page 6, line 6, please delete "12" and insert --14--,

Page 6, line 8, after "SEQ ID NOS." please delete "1, 4, 6, 9 or 12" and insert --3, 4, 8, 11 or 14--,

Page 6, line 15, after "SEQ ID NOS." please delete "1, 4, 6, 9 or 12" and insert --3, 4, 8, 11 or 14--,

Page 6, line 19, after "SEQ ID NOS." please delete "2, 5, 7, 10, 13, 3, 8, 11 or 14" and insert --1, 5, 6, 9, 12, 2, 7, 10 or 13--,

Page 6, line 26, after "SEQ ID NOS." please delete "2, 5, 7, 10, 13, 3, 8, 11 or 14" and insert --1, 5, 6, 9, 12, 2, 7, 10 or 13--,

Page 7, line 9, after "SEQ ID NOS." please delete "2, 5, 7, 10, 13, 3, 8, 11 or 14" and insert --1, 5, 6, 9, 12, 2, 7, 10 or 13--,

Page 8, line 7, after "SEQ ID NO." please delete "1" and insert --3--,

Page 8, line 11, after "SEQ ID NOS." please delete "1, 4, 6, 9" and insert --3, 4, 8, 11--,

Page 8, line 12, please delete "12" and insert --14--,

Page 8, line 20, after "NOS." please delete "1, 4, 6, 9 or 12" and insert --3, 4, 8, 11 or 14--,

Page 8, line 26, after "SEQ ID NOS." please delete "3, 8, 11" and insert --2, 7, 10--,

Page 8, line 27, please delete "14" and insert --13--,

Page 11, line 21, after "SEQ ID NOS." please delete "2, 5, 7, 10" and insert --1, 5, 6, 9--,

Page 11, line 22, please delete "13" and insert --12--,

Page 12, line 6, after "SEQ ID NOS." please delete "2, 5, 7, 10" and insert --1, 5, 6, 9--,

Page 12, line 7, please delete "13" and insert --12--,

Page 13, line 4, after "SEQ ID NOS." please delete "3, 8, 11 or 14" and insert --2, 7, 10 or 13--,

Page 13, line 19, after "SEQ ID NOS." please delete "2, 5, 7, 10 or 13" and insert --1, 5, 6, 9 or 12--,

Page 26, line 14, after the nucleic acid sequence, please insert --SEQ ID NO. 15--,

Page 27, line 25, after the nucleic acid sequence, please insert --SEQ ID NO. 16--,

Page 28, line 5, after "SEQ ID NO." please delete "3" and insert --2--,

Page 28, line 7, after "SEQ ID NOS." please delete "1, 2, 4 and 5" and insert --1, 3, 4 and 5--,

Page 28, line 19, after "SEQ ID NO." please delete "1" and insert --3--,

Page 29, line 4, after the nucleic acid sequence, please insert --SEQ ID NO. 17--,

Page 29, line 10, after "SEQ ID NO." please delete "8" and insert --7--,

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Page 29, line 12, after "SEQ ID NOS." please delete "6 and 7" and insert --6 and 8--,

Page 30, line 8, after the nucleic acid sequence, please insert --SEQ ID NO. 18--,

Page 30, line 17, after "SEQ ID NO." please delete "11" and insert --10--,

Page 30, line 19, after "SEQ ID NOS." please delete "9 and 10" and insert --9 and 11--,

Page 31, line 1, after "SEQ ID NO." please delete "9" and insert --11--,

Page 31, line 4, after "SEQ ID NO." please delete "9" and insert --11--,

Page 31, line 23, after the nucleic acid sequence, please insert --SEQ ID NO. 19--,

Page 32, line 1, after "SEQ ID NO." please delete "14" and insert --13--,

Page 32, line 3, after "SEQ ID NOS." please delete "12 and 13" and insert --12 and 14--,
and

Page 32, line 14, after "SEQ ID NO." please delete "12" and insert --14--.

IN THE CLAIMS:

Please amend the claims as follows:

Claim 1, line 2, after "SEQ ID NOS." please delete "1, 4, 6, 9 or 12" and insert --3, 4, 8, 11 or 14--,

Claim 2, line 2, after "SEQ ID NOS." please delete "1, 4, 6, 9 or 12" and insert --3, 4, 8, 11 or 14--,

Claim 4, line 2, after "SEQ ID NOS." please delete "2, 5, 7, 10 or 13" and insert --1, 5, 6, 9 or 12-- and

Claim 5, line 2, after "SEQ ID NOS." please delete "3, 8, 11 or 14" and insert --2, 7, 10 or 13--.

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REMARKS

A computer readable form of the Sequence Listing has not previously been filed in this application. The paper copy of the Sequence Listing filed with the application was prepared using PatentIn version 2.0. However, to comply with the recent rule changes, a computer readable form of the Sequence Listing, and substitute paper copy, was prepared using PatentIn 3.0. The later version of PatentIn automatically produces a separate peptide sequence when a coding sequence is identified in a polynucleotide sequence. As a result, two identical peptide sequences were generated for some of the sequences upon use of version 3.0, and the replicates had to be deleted. In addition, the numbering of the sequences in the specification and claims had to be changed. Thus, the amendments to the specification and claims are necessitated by the generation of the computer readable format of the Sequence Listing using PatentIn 3.0.

No new matter has been introduced.

Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,



Drew Hisson
Registration No. 44,765

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Date: January 5, 2001

DESCRIPTION

NOVEL POLYPEPTIDES, cDNA ENCODING THE SAME, AND USE OF THEM

Technical Field

The present invention relates to a novel polypeptide, a cDNA encoding it and a pharmaceutical use of it.

Technical Background

Until now, when a man skilled in the art intends to obtain a particular polypeptide or a cDNA encoding it, he generally utilizes methods by confirming an aimed biological activity in a tissue or in a cell medium, isolating and purifying the polypeptide and then cloning a gene or methods by "expression-cloning" with the guidance of the said biological activity. However, physiologically active polypeptides in living body have often many kinds of activities. Therefore, it happens increasingly that after cloning a gene, the isolated gene is found to be identical to that encoding a polypeptide already known. In addition, some factors could be generated in only a very slight amount and/or under specific conditions and it makes difficult to isolate and to purify the factor and to confirm its biological activity.

Recent rapid developments in techniques for constructing cDNAs and sequencing techniques have made it possible to quickly sequence a large amount of cDNAs. By utilizing these techniques, a process, which comprises constructing cDNAs library using various cells or tissues, cloning the cDNA at random, identifying the nucleotide sequences thereof, expressing novel polypeptides encoded by them, is now in progress. Although this process is advantageous in that a gene can be cloned and information regarding its nucleotide sequence can be obtained without any biochemical or genetic analysis, the target gene can be discovered thereby only accidentally in many cases.

Disclosure of the present invention

The present inventors have studied cloning method to isolate genes encoding proliferation and/or differentiation factors functioning in hematopoietic systems and immune systems. Focusing their attention on the fact that most of the secretory proteins such as proliferation and/or differentiation factors (for example various cytokines) and membrane proteins such as receptors thereof (hereafter these proteins will be referred to generally as secretory proteins and the like) have sequences called signal peptides in the N-termini, the inventors have conducted extensive studies on a process for efficiently and selectively cloning a gene encoding for a signal peptide. Finally, we have successfully developed a screening method for the signal peptides (signal sequence trap (SST)) by using mammalian cells (See Japanese Patent Application Kokai Hei 6-315380). We also developed yeast SST method on the same concept. By the method based on the same conception using yeast, (yeast SST method), genes including sequence encoding signal peptide can be identified more easily and efficiently (See USP No. 5, 536, 637).

The present inventors et al. have diligently performed certain investigation using the present invention in order to isolate novel factors (polypeptides) useful for treatment, diagnosis and/or study, particularly, secretory proteins containing secretory signal and membrane protein. From the result, the present inventors achieved to find novel secretory proteins and membrane proteins produced from cell lines and tissue, for example, human adult brain tissue, cell lines derived from human brain tissue and cell line derived from human bone marrow, and cDNAs encoding them, and then completed the present invention.

The present invention provides the cDNA sequences identified as clones OC001, OM237, OA004b which were isolated by the said yeast SST method using cDNA libraries prepared from human adult brain tissue and cell lines derived from human brain tissue (T98G, ATCC No. CRL-1690). Clones OC001, OM237, OA004b were full-length cDNA including full cDNA

sequences encoding membrain proteins (Each protein is represented as OC001, OM237, OA004b protein, respectively).

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequences of OC001, OM237, OA004b of the present invention. In addition, the polypeptides of the present invention were expected to possess the transmembrane region by hydrophobisity analysis of the obtained amino acid sequences. From these results, it was proved that polypeptides OC001, OM237, OA004b of the present invention were new membrane proteins.

The present invention provides the cDNA sequence identified as clone OAF075b which was isolated by the said yeast SST method using cDNA libraries prepared from human bone marrow cell line HAS303 (human bone marrow cell line: provided from Prof. Keisuke Sotoyama, Dr. Makoto Aizawa, First Medicine, Tokyo Medical College. see J. Cell. Physiol. 148, 245-251, 1991 and Experimental Hematol. 22, 482-487, 1994). Clone OAF075b was a full-length cDNA including a full cDNA sequence encoding secretory protein (this protein is represented as OAF075b protein).

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OAF075b of the present invention. In addition, the polypeptide of the present invention was expected to possess no transmembrane region by hydrophobisity analysis of the obtained amino acid sequence. From these results, it was proved that polypeptide of the present invention was a new secretory protein.

The present invention relates to

- (1) a polypeptide comprising an amino acid sequence of SEQ ID NOS. 1, 4,

6, 9 or 12,

(2) a cDNA encoding the polypeptide described in (1),

(3) a cDNA comprising a nucleotide sequence of SEQ ID NOS. 2, 5, 7, 10 or 13, and

(4) a cDNA comprising a nucleotide sequence of SEQ ID NOS. 3, 8, 11 or 14.

Detailed Description of the present invention

The present invention relates to a substantially purified form of the polypeptide comprising the amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12, homologue thereof, fragment thereof or homologue of the fragment.

Further, the present invention relates to cDNAs encoding the above peptides. More particularly the invention is provided cDNAs comprising nucleotide sequence shown in SEQ ID NOS. 2, 5, 7, 10 or 13, and cDNA containing a fragment which is selectively hybridizing to the cDNA comprising nucleotide sequence shown in SEQ ID NOS. 2, 5, 7, 10, 13, 3, 8, 11 or 14. A said cDNA capable for hybridizing to the cDNA includes the contemporary sequence of the above sequence.

A polypeptide comprising amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12 in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is that of the SEQ ID NOS. 1, 4, 6, 9 or 12.

A homologue of polypeptide comprising amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the polypeptide comprising the said amino acid sequence over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 more contiguous amino acids. Such a polypeptide homologue will be referred to a polypeptide of the present invention.

Further, a fragment of polypeptide comprising amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12 or its homologues will be at least 10, preferably at least 15, for example 20, 25, 30, 40, 50 or 60 amino acids in length.

A cDNA capable of selectively hybridizing to the cDNA comprising nucleotide sequence shown in SEQ ID NOS. 2, 5, 7, 10, 13, 3, 8, 11 or 14 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the cDNA comprising the said nucleotide sequence over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such a cDNA will be referred to "a cDNA of the present invention".

Fragments of the cDNA comprising nucleotide sequence shown in SEQ ID NOS. 2, 5, 7, 10, 13, 3, 8, 11 or 14 will be at least 10, preferably at least 15, for example 20, 25, 30 or 40 nucleotides in length, and will be also referred to "a cDNA of the present invention" as used herein.

A further embodiment of the present invention provides replication and expression vectors carrying cDNA of the present invention. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said cDNA and optionally a regulator of the promoter. The vector may contain one or more selectable marker genes, for example ampicillin resistance gene. The vector may be used in vitro, for example of the production of RNA corresponding to the cDNA, or used to transfect a host cell.

A further embodiment of the present invention provides host cells transformed with the vectors for the replication and expression of the cDNA of the present invention, including the cDNA comprising nucleotide sequence shown in SEQ ID NOS. 2, 5, 7, 10, 13, 3, 8, 11 or 14 or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect cells or mammalian cells.

A further embodiment of the present invention provides a method of producing a polypeptide which comprises culturing host cells of the present

invention under conditions effective to express a polypeptide of the present invention. Preferably, in addition, such a method is carried out under conditions in which the polypeptide of the present invention is expressed and then produced from the host cells.

cDNA of the present invention may also be inserted into the vectors described above in an antisense orientation in order to prove for the production of antisense RNA. Such antisense RNA may be used in a method of controlling the levels of a polypeptide of the present invention in a cell.

The invention also provides monoclonal or polyclonal antibodies against a polypeptide of the present invention. The invention further provides a process for the production of monoclonal or polyclonal antibodies to the polypeptides of the present invention. Monoclonal antibodies may be prepared by common hybridoma technology using polypeptides of the present invention or fragments thereof, as an immunogen. Polyclonal antibodies may also be prepared by common means which comprise inoculating host animals, (for example a rat or a rabbit etc.), with polypeptides of the present invention and recovering immune serum.

The present invention also provides pharmaceutical compositions containing a polypeptide of the present invention, or an antibody thereof, in association with a pharmaceutically acceptable diluent and/or carrier.

The polypeptide of the present invention specified in (1) includes that which a part of their amino acid sequence is lacking (e.g., a polypeptide comprised of the only essential sequence for revealing a biological activity in an amino acid sequence shown in SEQ ID NO. 1), that which a part of their amino acid sequence is replaced by other amino acids (e. g., those replaced by an amino acid having a similar property) and that which other amino acids are added or inserted into a part of their amino acid sequence, as well as those comprising the amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12.

As known well, there are one to six kinds of codon as that encoding

one amino acid (for example, one kind of codon for Methionine (Met), and six kinds of codon for Leucine (Leu) are known). Accordingly, the nucleotide sequence of cDNA can be changed in order to encode the polypeptide having the same amino acid sequence.

The cDNA of the present invention, specified in (2) includes a group of every nucleotide sequence encoding polypeptides (1) shown in SEQ ID NOS. 1, 4, 6, 9 or 12. There is a probability that yield of a polypeptide is improved by changing a nucleotide sequence.

The cDNA specified in (3) is the embodiment of the cDNA shown in (2), and indicate the sequence of natural form.

The cDNA shown in (4) indicates the sequence of the cDNA specified in (3) with natural non-translational region.

cDNA carrying nucleotide sequence shown in SEQ ID NOS. 3, 8, 11 or 14 is prepared by the following method:

Brief description of Yeast SST method (see USP No. 5, 536, 637) is as follows.

Yeast such as *Saccharomyces cerevisiae* should secrete invertase into the medium in order to take sucrose or raffinose as a source of energy or carbon. (Invertase is an enzyme to cleave raffinose into sucrose and melibiose, sucrose into fructose and glucose). It is known that many known mammalian signal sequence make yeast secrete its invertase. From these knowledge, SST method was developed as a screening method to find novel signal peptide which make it possible can to secrete yeast invertase from mammalian cDNA library. SST method uses yeast growth on raffinose medium as a marker. Non-secretory type invertase gene SUC2 (GENBANK Accession No. V 01311) lacking initiation codon ATG was inserted to yeast expression vector to prepare yeast SST vector pSUC2. In this expression vector, ADH promoter, ADH terminator (both were derived from AAH5 plasmid (Gammerer, Methods in Enzymol. 101, 192-201, 1983)), 2 μ ori (as a yeast replication origin), TRP1 (as a yeast selective marker), ColE1 ori (as a E. Coli replication origin) and ampicillin resistance gene (as

a drug resistance marker) were inserted. Mammalian cDNA was inserted into the upstream of SUC2 gene to prepare yeast SST cDNA library. Yeast lacking secretory type invertase, was transformed with this library. If inserted mammalian cDNA encodes a signal peptide, yeast could survive in raffinose medium as a result of restoring secretion of invertase. Only to culture yeast colonies, prepare plasmids and determine the nucleotide sequence of the insert cDNAs, it is possible to identify novel signal peptide rapidly and easily.

Preparation of yeast SST cDNA library is as follows:

- (1) mRNA is isolated from the targeted cells, double-strand synthesis is performed by using random primer with certain restriction enzyme (enzyme I) recognition site,
- (2) obtained double-strand cDNA is ligated to adapter containing certain restriction endonuclease (enzyme II) recognition site, differ from enzyme I, digested with enzyme I and fractionated in a appropriate size,
- (3) obtained cDNA fragment is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted and the library was transformed.

Detailed description of each step is as follows:

- (1) mRNA is isolated from mammalian organs and cell lines stimulate them with appropriate stimulator if necessary) by known methods (Molecular Cloning (Sambrook, J., Fritsch, E. F. and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989) or Current Protocol in Molecular Biology (F. M. Ausubel et al, John Wiley & Sons, Inc) if not remark especially).

HAS303 (human bone marrow stroma cell line: provide from Professor Keisuke Sotoyama, Dr. Makoto Aizawa of First Medicine, Tokyo Medical College; see J. Cell. Physiol., 148, 245-251, 1991 and Experimental Hematol., 22, 482-487, 1994) or human glioblastoma cell line TG98G (ATCC No. CRL-1690) are chosen as a cell line. Human adult brain is chosen as a tissue source. Double-strand cDNA synthesis using random primer is performed by known methods.

Any sites may be used as restriction endonuclease recognition site I which is linked to adapter and restriction endonuclease recognition site II which is used in step (2), if both sites are different each other. Preferably, XhoI is used as enzyme I and EcoRI as enzyme II.

In step (2), cDNA is created blunt-ends with T4 DNA polymerase, ligated enzyme II adapter and digested with enzyme I. Fragment cDNA is analyzed with agarose-gel electrophoresis (AGE) and is selected cDNA fraction ranging in size from 300 to 800 bp. As mentioned above, any enzyme may be used as enzyme II if it is not same the enzyme I.

In step (3), cDNA fragment obtained in step (2) is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted. E. Coli was transformed with the expression vector. Many vectors are known as yeast expression plasmid vector. For example, YEp24 is also functioned in E. Coli. Preferably pSUC2 as described above is used.

Many host E. Coli strains are known for transformation, preferably DH10B competent cell is used. Any known transformation method is available, preferably it is performed by electroporation method. Transformant is cultured by conventional methods to obtain cDNA library for yeast SST method.

However not every all of the clones do not contain cDNA fragment. Further all of the gene fragments do not encode unknown signal peptides. It is therefore necessary to screen a gene fragment encoding for an unknown signal peptide from the library.

Therefore, screening of fragments containing a sequence encoding an appropriate signal peptide is performed by transformation of the cDNA library into *Saccharomyces cerevisiae* (e. g. Y455 strain) which lack invertase (it may be prepared by known methods).

Transformation of yeast is performed by known methods, e. g. lithium acetate method. Transformant is cultured in a selective medium, then transferred to a medium containing raffinose as a carbon source.

Survival colonies are selected and then prepared plasmid. Survival colonies on a raffinose-medium indicates that some signal peptide of secretory protein was inserted to this clone.

As for isolated positive clones, the nucleotide sequence is determined. As a cDNA encodes unknown protein, full-length clone may be isolated by using cDNA fragment as a probe and then determined to obtain full-length nucleotide sequence. These manipulation is performed by known methods.

Once the nucleotide sequences shown in SEQ ID NO. 2, 5, 7, 10 or 13 are determined partially or preferably fully, it is possible to obtain DNA encode mammalian protein itself, homologue or subset. cDNA library or mRNA derived from mammals was screened by PCR with any synthesized oligonucleotide primers or by hybridization with any fragment as a probe. It is possible to obtain DNA encodes other mammalian homologue protein from other mammalian cDNA or genome library.

If a cDNA obtained above contains a nucleotide sequence of cDNA fragment obtained by SST (or consensus sequence thereof), it will be thought that the cDNA encodes signal peptide. So it is clear that the cDNA will be full-length or almost full. (All signal peptides exist at N-termini of a protein and are encoded at 5'-termini of open reading frame of cDNA)

The confirmation may be carried out by Northern analysis with the said cDNA as a probe. It is thought that the cDNA is almost complete length, if length of the cDNA is almost the same length of the mRNA obtained in the hybridizing band.

Once the nucleotide sequences shown in SEQ ID NOS. 2, 5, 7, 10 or 13 are determined, DNAs of the invention are obtained by chemical synthesis, or by hybridization making use of nucleotide fragments which are chemically synthesized as a probe. Furthermore, DNAs of the invention are obtained in desired amount by transforming a vector that contains the DNA into a proper host, and culturing the transformant.

The polypeptides of the present invention may be prepared by:

(1) isolating and purifying from an organism or a cultured cell,
(2) chemically synthesizing, or
(3) using recombinant cDNA technology,
preferably, by the method described in (3) in an industrial production.

Examples of expression system (host-vector system) for producing a polypeptide by using recombinant cDNA technology are the expression systems of bacteria, yeast, insect cells and mammalian cells.

In the expression of the polypeptide, for example, in *E. Coli*, the expression vector is prepared by adding the initiation codon (ATG) to 5' end of a cDNA encoding mature peptide, connecting the cDNA thus obtained to the downstream of a proper promoter (e. g., trp promoter, lac promoter, λ PL promoter, T7 promoter etc.), and then inserting it into a vector (e. g., pBR322, pUC18, pUC19 etc.) which functions in an *E. Coli* strain.

Then, an *E. Coli* strain (e. g., *E. Coli* DH1 strain, *E. Coli* JM109 strain, *E. Coli* HB101 strain, etc.) which is transformed with the expression vector described above may be cultured in a appropriate medium to obtain the desired polypeptide. When a signal sequence of bacteria (e. g., signal sequence of pel B) is utilized, the desired polypeptide may be also released in periplasm. Furthermore, a fusion protein with other polypeptide may be also produced readily.

In the expression of the polypeptide, for example, in a mammalian cells, for example, the expression vector is prepared by inserting the cDNA encoding nucleotide shown in SEQ ID NOS. 3, 8, 11 or 14 into the downstream of a proper promoter (e. g., SV40 promoter, LTR promoter, metallothionein promoter etc.) in a proper vector (e. g., retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40 vector, etc.). A proper mammalian cell (e. g., monkey COS-7 cell, Chinese hamster CHO cell, mouse L cell etc.) is transformed with the expression vector thus obtained, and then the transformant is cultured in a proper medium to express the aimed secretory protein and membrane protein of the present invention by the following method.

In case of secretory protein as for the present invention, the aimed polypeptide was expressed in the supernatant of the cells. In addition, fusion protein may be prepared by conjugating cDNA fragment encoding the other polypeptide, for example, Fc portion of antibody.

On the other hand, in case of membrane protein as for the present invention, the aimed polypeptide was expressed on the cell membrane. A cDNA encoding the nucleotide sequence of SEQ ID NOS. 2, 5, 7, 10 or 13 with deletion of extracellular region was inserted into the said vector, transfected into the an adequate mammalian cells to secret the aimed soluble polypeptide in the culture medium. In addition, fusion protein may be prepared by conjugating cDNA fragment encoding the said mutant with deletion of extracellular region and other polypeptide, for example, Fc portion of antibody.

The polypeptide available by the way described above can be isolated and purified by conventional biochemical method.

Industrial Applicability

It is considered that the polypeptide of the present invention and a cDNA which encodes the polypeptide will show one or more of the effects or biological activities (including those which relates to the assays cited below) The effects or biological activities described in relation to the polypeptide of the present invention are provided by administration or use of the polypeptide or by administration or use of a cDNA molecule which encodes the polypeptide (e. g., vector suitable for gene therapy or cDNA introduction).

[Cytokine activity and cell proliferation/differentiation activity]

The protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent

cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a polypeptide of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines.

[Immune stimulating/suppressing activity]

The protein of the present invention may also exhibit immune stimulating or immune suppressing activity. The protein of the present invention may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e. g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral infection such as HIV as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using the polypeptide of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leishmania, malaria and various fungal infections such as candida. Of course, in this regard, the protein of the present invention may also be useful where a boost to the immune system generally would be indicated, i. e., in the treatment of cancer.

The protein of the present invention may be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems. The protein of the present invention may also be useful in the treatment of the other conditions required to suppress the immune system (for example, asthma or respiratory disease)

The protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection such as septic shock or systemic inflammatory response syndrome (SIRS), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1 wherein the effect was

demonstrated by IL- 11.

[Hematopoiesis regulating activity]

The protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis. The said biological activities are concerned with the following all or some example(s). e. g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemia or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i. e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vitro or ex-vivo (i. e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

The suitable method of assay for proliferation and differentiation of various hematopoietic stem cell lines is described above.

The activity of the protein of the present invention may, among other

means, be measured by the following methods :

[Tissue generation/regeneration activity]

The protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, Ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair, and in the treatment of burns, incisions and ulcers.

The protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, may be applied to the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing the protein of the present invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

The protein of the present invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. The protein of the present invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. The protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, may be applied to the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans

and other animals. Such a preparation employing the protein inducing a tendon/Ligament-like tissue may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon Ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the present invention may also be useful in the treatment of tendinitis, Carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue. i. e. for the treatment of central and peripheral nervous system diseases and neuropathies. as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, the protein of the present invention may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical

therapies may also be treatable using the polypeptide of the present invention.

It is expected that the protein of the present invention may also exhibit activity for generation of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the proliferation of cells comprising such tissues. Part of the desired effects may be by inhibition of fibrotic scarring to allow normal tissue to regenerate.

The protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

[Activin/Inhibin activity]

The protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, the protein of the present invention alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the present invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary (See USP 4, 798, 885). The protein of the present invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as

cows, sheep and pigs.

[Chemotactic/chemokinetic activity]

The protein of the present invention may have chemotactic or chemokinetic activity e. g., functioning as a chemokine, for mammalian cells, including, for example, monocytes, neutrophils, T-cells, mast cells, eosinophils and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

If a protein or peptide can stimulate, directly or indirectly, the directed orientation or movement of such cell population, it has chemotactic activity for a particular cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

[Hemostatic and thrombolytic activity]

The protein of the present invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the present invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom such as, for example, infarction or stroke.

[Receptor/ligand activity]

The protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including cellular adhesion molecules such as Selectins, Integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. The protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

[Other activity]

The protein of the present invention may also exhibit one or more of the following additional activities or effects: inhibiting growth of or killing the infecting agents including bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) body characteristics including height, weight, hair color, eye color, skin, other tissue pigmentation, or organ or body part size or shape such as, for example, breast augmentation or diminution etc.; effecting elimination of dietary fat, protein, carbohydrate; effecting behavioral characteristics including appetite, libido, stress, cognition (including cognitive disorders), depression and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases.

The protein with above activities, is suspected to have following functions by itself or interaction with its ligands or receptors or association

with other molecules. For example, proliferation or cell death of B cells, T cells and/or mast cells; specific induction by promotion of class switch of immunoglobulin genes; differentiation of B cells to antibody-forming cells; proliferation, differentiation, or cell death of precursors of granulocytes; proliferation, differentiation, or cell death of precursors of monocytes-macrophages; proliferation, of up regulation or cell death of neutrophils, monocytes-macrophages, eosinophils and/or basophils; proliferation, or cell death of precursors of megakaryocytes; proliferation, differentiation, or cell death of precursors of neutrophils; proliferation, differentiation, or cell death of precursors of T cells and B cells; promotion of production of erythrocytes; sustainment of proliferation of erythrocytes, neutrophils, eosinophils, basophils, monocytes-macrophages, mast cells, precursors of megakaryocyte; promotion of migration of neutrophils, monocytes-macrophages, B cells and/or T cells; proliferation or cell death of thymocytes; suppression of differentiation of adipocytes; proliferation or cell death of natural killer cells; proliferation or cell death of hematopoietic stem cells; suppression of proliferation of stem cells and each hematopoietic precursor cells; promotion of differentiation from mesenchymal stem cells to osteoblasts or chondrocytes, proliferation or cell death of mesenchymal stem cells, osteoblasts or chondrocytes and promotion of bone absorption by activation of osteoclasts and promotion of differentiation from monocytes to osteoclasts.

The polypeptide of the present invention is also suspected to function to nervous system, so expected to have functions below; differentiation to kinds of neurotransmitter-responsive neurons, survival or cell death of these cells; promotion of proliferation or cell death of glial cells; spread of neural dendrites; survival or cell death of gangriocytes; proliferation, promotion of differentiation, or cell death of astrocytes; proliferation, survival or cell death of peripheral neurons; proliferation or cell death of Schwann cells; proliferation, survival or cell death of motoneurons.

Furthermore, in the process of development of early embryonic, the polypeptide of the present invention is expected to promote or inhibit the

organogenesis of epidermis, brain, backbone, and nervous system by induction of ectoderm, that of notochord connective tissues (bone, muscle, tendon), hemocytes, heart, kidney, and genital organs by induction of mesoderm, and that of digestive apparatus (stomach, intestine, liver, pancreas), respiratory apparatus (lung, trachea) by induction of endoderm. In adult, also, this polypeptide is thought to proliferate or inhibit the above organs.

Therefore, the polypeptide of the present invention itself is expected to be used as an agent for the prevention or treatment of disease of progression or suppression of immune, nervous, or bone metabolic function, hypoplasia or overgrowth of hematopoietic cells: for example, inflammatory disease (rheumatism, ulcerative colitis, etc.), decrease of hematopoietic stem cells after bone marrow transplantation, decrease of leukocytes, platelets, B-cells, or T-cells after radiation exposure or chemotherapeutic dosage against cancer or leukemia, anemia, infectious disease, cancer, leukemia, AIDS, bone metabolic disease (osteoporosis etc.), various degenerative disease (Alzheimer's disease, multiple sclerosis, etc.), or nervous lesion.

In addition, since the polypeptide of the present invention is thought to induce the differentiation or growth of organs derived from ectoderm, mesoderm, and endoderm, this polypeptide is expected to be an agent for tissue repair (epidermis, bone, muscle, tendon, heart, kidney, stomach, intestine, liver, pancreas, lung, and trachea, etc.).

By using polyclonal or monoclonal antibodies against the polypeptide of the present invention, quantitation of the said polypeptide in the body can be performed. It can be used in the study of relationship between this polypeptide and disease or diagnosis of disease, and so on. Polyclonal and monoclonal antibodies can be prepared using this polypeptide or its fragment as an antigen by conventional methods.

Identification, purification or molecular cloning of known or unknown proteins which bind the polypeptide of the present invention (preferably polypeptide of extracellular domain) can be performed using the polypeptide

of the present invention by, for example, preparation of the affinity-column.

Identification of the downstream signal transmission molecules which interact with the polypeptide of the present invention in cytoplasm and molecular cloning of the gene can be performed by west-western method using the polypeptide of the present invention (preferably polypeptide of transmembrane region or intracellular domain), or by yeast two-hybrid system using the cDNA (preferably cDNA encoding transmembrane region or cytoplasmic domain of the polypeptide).

Agonists/antagonists of this receptor polypeptide and inhibitors between receptor and signal transduction molecules can be screened using the polypeptide of the present invention.

cDNAs of the present invention are useful not only the important and essential template for the production of the polypeptide of the present invention which is expected to be largely useful, but also be useful for diagnosis or therapy (for example, treatment of gene lacking, treatment to stop the expression of the polypeptide by antisense cDNA (mRNA)). Genomic cDNA may be isolated with the cDNA of the present invention, as a probe. As the same manner, a human gene encoding which can be highly homologous to the cDNA of the present invention, that is, which encodes a polypeptide highly homologous to the polypeptide of the present invention and a gene of animals excluding mouse which can be highly homologous to the cDNA of the present invention, also may be isolated.

[Application to Medicaments]

The polypeptide of the present invention or the antibody specific for the polypeptide of the present invention is administered systemically or topically and in general orally or parenterally, preferably parenterally, intravenously and intraventricularly, for preventing or treating the said diseases.

The doses to be administered depend upon age, body weight, symptom, desired therapeutic effect, route of administration, and duration of

the treatment etc. In human adults, one dose per person is generally between 100 μ g and 100 mg, by oral administration, up to several times per day, and between 10 μ g and 100 mg, by parental administration up to several times per day.

As mentioned above, the doses to be used depend upon various conditions. Therefore, there are cases in which doses lower than or greater than the ranges specified above may be used.

The compounds of the present invention, may be administered as solid compositions, liquid compositions or other compositions for oral administration, as injections, liniments or suppositories etc. for parental administration.

Solid compositions for oral administration include compressed tablets, pills, capsules, dispersible powders, and granules. Capsules include soft or hard capsules.

In such compositions, one or more of the active compound(s) is or are admixed with at least one inert diluent (such as lactose, mannitol, glucose, hydroxypropyl cellulose, microcrystalline cellulose, starch, polyvinylpyrrolidone, magnesium metasilicate aluminate, etc.). The compositions may also comprise, as is normal practice, additional substances other than inert diluents: e. g. lubricating agents (such as magnesium stearate etc.), disintegrating agents (such as cellulose calcium glycolate, etc.), stabilizing agents (such as human serum albumin, lactose etc.), and assisting agents for dissolving (such as arginine, asparaginic acid etc.).

The tablets or pills may, if desired, be coated with a film of gastric or enteric materials (such as sugar, gelatin, hydroxypropyl cellulose or hydroxypropylmethyl cellulose phthalate, etc.), or be coated with more than two films. And then, coating may include containment within capsules of absorbable materials such as gelatin.

Liquid compositions for oral administration include pharmaceutically-acceptable emulsions, solutions, syrups and elixirs. In such compositions, one or more of the active compound(s) is or are contained in inert diluent(s)

commonly used (purified water, ethanol etc.). Besides inert diluents, such compositions may also comprise adjuvants (such as wetting agents, suspending agents, etc.), sweetening agents, flavoring agents, perfuming agents, and preserving agents.

Other compositions for oral administration include spray compositions which may be prepared by known methods and which comprise one or more of the active compound(s). Spray compositions may comprise additional substances other than inert diluents: e. g. stabilizing agents (sodium sulfite etc.), isotonic buffer (sodium chloride, sodium citrate, citric acid, etc.). For preparation of such spray compositions, for example, the method described in the United States Patent No. 2, 868, 691 or 3, 095, 355 (herein incorporated in their entireties by reference) may be used.

Injections for parental administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. In such compositions, one or more active compound(s) is or are admixed with at least one inert aqueous diluent(s) (distilled water for injection, physiological salt solution, etc.) or inert non-aqueous diluents(s) (propylene glycol, polyethylene glycol, olive oil, ethanol, POLYSOLBATE 80 (Trade mark) etc.).

Injections may comprise additional compound other than inert diluents: e. g. preserving agents, wetting agents, emulsifying agents, dispersing agents, stabilizing agent (such as human serum albumin, lactose, etc.), and assisting agents such as assisting agents for dissolving (arginine, asparaginic acid, etc.).

Best Mode carrying out the Invention

The invention is illustrated by the following examples relating to clone OC001 of the present invention, but not limit the invention.

Example 1: Preparation of Poly(A)⁺RNA

Total RNA was prepared from human placenta tissue by TRIzol reagent (Trade Mark, marketed from GIBCOBRL Co.). Poly(A)⁺RNA was

purified from the total RNA by mRNA Purification Kit (Trade name, marketed from Pharmacia Co.).

Example 2: Preparation of yeast SST cDNA library

Double strand cDNA was synthesized by Super Script Plasmid System for cDNA Synthesis and Plasmid Cloning (Trade name, marketed from GIBCOBRL Co.) with above poly(A)⁺RNA as template and random 9mer as primer which was containing XhoI site:

5'-CGATTGAATTCTAGACCTGCCTCGAGNNNNNNNNN-3'
(SEQ ID NO. 15).

cDNA was ligated EcoRI adapter by DNA ligation kit ver. 2 (Trade name, marketed from Takara-Shuzo Co., this kit was used in all ligating steps hereafter) and digested by XhoI. cDNAs were separated by agarose-gel electrophoresis. 300~800 bp cDNAs were isolated and were ligated to EcoRI/NotI site of pSUC2 (see US Patent No. 5, 536, 637). E. Coli DH10B strains were transformed by pSUC2 with electroporation to obtain yeast SST cDNA library.

Example 3: Screening by SST method and determination of nucleotide sequence of SST positive clone

Plasmids of the said cDNA library were prepared. Yeast YTK12 strains were transformed by the plasmids with lithium acetate method (Current Protocols In Molecular Biology 13.7.1). The transformed yeast were plated on triptphan-free medium (CMD-Trp medium) for selection. The plate was incubated for 48 hour at 30 °C. Replica of the colony (transformant) which was obtained by Accutran Replica Plater (Trade name, marketed from Schleicher & Schuell Co.) were placed onto YPR plate containing raffinose for carbon source, and the plate was incubated for 14 days at 30°C. After 3 days, each colony appeared was streaked on YPR plate again. The plates were incubated for 48 hours at 30°C. Single colony was inoculated to YPD medium and was incubated for 48 hours at 30°C.

Then plasmids were prepared. Insert cDNA was amplified by PCR with two kind primers which exist end side of cloning site on pSUC2 (sense strand primers were biotinylated). Biotinylated single strand of cDNAs were purified with Dynabeads (Trade name, marketed from DYNAL Co.) and the nucleotide sequences were determined. Sequencing was performed by Dye Terminator Cycle Sequencing Ready Reaction with DNA Sequencing kit (Trade name, marketed from Applied Biosystems Inc.) and sequence was determined by DNA sequencer 373 (Applied Biosystems Inc.) (All sequencing hereafter was carried out with this method).

We tried to carry out cloning of full-length cDNA which was proved to be new one according to the homology search for the obtained nucleotide sequences and deduced amino acid sequences in data base.

Example 4: Cloning of a full-length cDNA and determination of nucleotide sequence of clone OC001

A full-length cDNA was cloned using Marathon cDNA Amplification Kit (Trade name, marketed from Clontech Co.) according to 3' RACE (Rapid Amplification of cDNA End) method. I. e., poly (A)*RNA in human adult brain tissue 27mer primer OC001-F1:

5'-GTCCTTCAGCAAAACAGTGGATTAAA-3' (SEQ ID NO. 16)

containing the deduced initiation ATG codon region based on the information of nucleotide sequence obtained by SST, was prepared. PCR was performed with the said primer and adapter primer attached in the kit. A cDNA which was amplified with clone OC001 specifically, was separated with agarose-gel electrophoresis, ligated to pT7 Blue-2 T-Vector (Trade name, marketed from Novagen Co) and transfected into E. Coli DH5 α to prepare the plasmid. Nucleotide sequences of 5'-end were determined, and the existence of nucleotide sequence OC001 SST cDNA was confirmed. Nucleotide sequence of full-length OC001 SST cDNA was determined and then sequence shown in SEQ ID NO. 3 was obtained. An open reading frame was determined and deduced amino acid sequence and nucleotide

sequence shown in SEQ ID NOS. 1, 2, 4 and 5, respectively, were obtained.

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OC001 of the present invention. In addition, the polypeptide of the present invention was expected to possess the transmembrane region at C-terminal and to be GPI anchor type by hydrophobisity analysis of the obtained amino acid sequence. From these results, it was proved that polypeptide of the present invention was new membrane protein. Further, the search using BLASTX, BLASTP and FASTA revealed a significant homology between clone OC001 (region of 12th~344th amino acid in SEQ ID NO. 1) and neurotrimin [*Rattus norvegicus*] (region of 9th~344th amino acid of Genbank Accession U16845) and opioid-binding cell adhesion molecule [*Homo sapiens*] (region of 9th~345th amino acid of Genbank Accession L34774). Based on these homologies, clone OC001 and nervous cell adhesion molecule family including neurotrimin and opioid-binding cell adhesion molecule were expected to share at least some activity.

Example 5: Cloning of a full-length cDNA and determination of nucleotide sequence of clone OM237

In Example relating to clone OM237 of the present invention, the same procedure as in Example of OC001 was used except for the following points.

A full-length cDNA was cloned by the same procedure as OC001 using Marathon cDNA Amplification Kit (Trade name, marketed from Clontech Co.) according to 3'RACE. A double-strand cDNA was prepared from RNA derived from each clone, i. e., poly(A)⁺RNA of human adult brain tissue. 27mer primer OM237-F1:

5'-CCAGAAAGCACAGCCCTGATTCTGCGT-3' (SEQ ID NO. 17)
containing the deduced initiation ATG codon region based on the information

of nucleotide sequence obtained by SST, was prepared. PCR was performed with the said primer and adapter primer attached in the kit. A cDNA which was amplified with clone OM237 specifically, was recloned by the same method as OC001 to determine full nucleotide sequence and obtain the sequence shown in SEQ ID NO. 8. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 6 and 7, respectively, were obtained.

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OM237 of the present invention. In addition, the polypeptide of the present invention was expected to possess the transmembrane region by hydrophobicity analysis of the obtained amino acid sequence. From these results, it was proved that polypeptide of the present invention was new membrane protein.

Example 6: Cloning of a full-length cDNA and determination of nucleotide sequence of clone OA004b

In Example relating to clone OA004b of the present invention, the same procedure as in Example of OC001 was used except for the following points.

[preparation of poly(A)⁺RNA]

Total RNA was prepared from human glioblastoma cell line T98G (ATCC No. CRL-1690) by TRIzol reagent (Trade Mark, marketed from GIBCOBRL Co.). Poly(A)⁺RNA was purified from total RNA by mRNA Purification Kit (Trade name, marketed from Pharmacia Co.).

[Cloning of a full-length cDNA and determination of amino acid sequence]

A full-length cDNA was cloned by GENETRAPPER cDNA Positive Selection System (GIBCOBRL Co.). First, dT-primed cDNA library was

prepared using plasmid pSPORT1 (GIBCOBRL Co.) as a vector from poly(A)⁺RNA of human glioblastoma cell line T98G by Super Script Plasmid System for cDNA Synthesis and Plasmid Cloning (Trade name, marketed from GIBCOBRL Co.). After preparing 27mer biotinylated primer OA004-F1:

5'-biotin-ATGCACATCTTCAAGCATGCTCAG-3' (SEQ ID NO. 18), based on the information of nucleotide sequence obtained by SST, plasmid hybridized specifically with the biotinylated primer were recovered from the cDNA library according to the method of Gene Trapper Kit and then transfected into E. Coli DH10B. Colony hybridization with OA004 SST cDNA which was labeled with ³²P-dCTP, as a probe, was performed by using Random Primer DNA Labeling kit (Trade name, marketed from Takara-Shuzo Co.) according to known method to isolate the positive clone and to prepare the plasmid. Full Nucleotide sequences was determined, and then sequence shown in SEQ ID NO. 11, which was named as OA004b, was obtained. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 9 and 10, respectively, were obtained.

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OA004b of the present invention. In addition, the polypeptide of the present invention was expected to possess the transmembrane region by hydrophobicity analysis of the obtained amino acid sequence. From these results, it was proved that polypeptide of the present invention was new membrane protein. However, the search using BLASTX, BLASTP and FASTA revealed a significant homology between clone OA004b (region of 171st~311st amino acid in SEQ ID NO. 9) and Hypothetical 52.8kD protein [*Caenorhabditis elegans*] (region of 299th~453rd amino acid of Swiss Prot Accession YJ95_CAEEL), and between

OA004b (region of 194th~277th amino acid in SEQ ID NO. 9) and presenilin-2 [Homo sapiens] (region of 340th~416th amino acid of Genbank Accession A56993). Based on these homologies, clone OA004b and presenilin family were expected to share at least some activity.

Example 7: Cloning of a full-length cDNA and determination of nucleotide sequence of clone OAF075b

In Example relating to clone OAF075b of the present invention, the same procedure as in Example of OC001 was used except for the following points.

[Preparation of poly(A)⁺RNA]

Total RNA was prepared from human bone marrow stroma cell line HAS303 (provided from Prof. Keisuke Sotoyama, Dr. Makoto Aizawa, First Medicine, Tokyo Medical College) by TRIzol reagent (Trade Mark, marketed from GIBCOBRL Co.). Poly(A)⁺RNA was purified from the total RNA by mRNA Purification Kit (Trade name, marketed from Pharmacia Co.).

[Cloning of a full-length cDNA and determination of amino acid sequence]

A full-length cDNA was cloned by the same procedure as OC001 using Marathon cDNA Amplification Kit (Trade name, marketed from Clontech Co.) according to 3'RACE. A double-strand cDNA was prepared from RNA derived from each clone, i. e., poly(A)⁺RNA of HAS303. 27mer primer OAF075-F1:

5'-CCCCGGGGACATGAGGTGGATACTGTT-3' (SEQ ID NO. 19)

containing the deduced initiation ATG codon region based on the information of nucleotide sequence obtained by SST, was prepared. PCR was performed with the said primer and adapter primer attached in the kit. A cDNA which was amplified with clone OAF075B specifically, was recloned by the same method as OC001 to determine full nucleotide sequence and obtain the sequence shown in SEQ ID NO. 14, which was named as OAF075b. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 12 and 13, respectively, were

obtained.

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OAF075b of the present invention.

In addition, the polypeptides of the present invention was expected to possess no transmembrane region by hydrophobicity analysis of the obtained amino acid sequences. From these results, it was proved that polypeptide of the present invention was new secretory protein. Further, the search using BLASTX, BLASTP and FASTA revealed a significant homology between clone OAF075b (region of 1st~359th amino acid in SEQ ID NO. 12) and preprocarboxypeptidase A2 [Homo sapiens] (region of 1st~355th amino acid of Genbank Accession U19977). Based on these homologies, clone OAF075b and preprocarboxypeptidase A2 [Homo sapiens] were expected to share at least some activity.

Claim

1. A substantially purified form of the polypeptide comprising the amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12, homologue thereof, fragment thereof or homologue of the fragment.
2. A polypeptide according to claim 1 comprising the amino acid sequence shown in SEQ ID NOS. 1, 4, 6, 9 or 12.
3. A cDNA encoding the polypeptide according to claim 1.
4. A cDNA according to claim 3 comprising the nucleotide sequence shown in SEQ ID NOS. 2, 5, 7, 10 or 13, or a fragment cDNA selectively hybridized to the cDNA.
5. A cDNA according to claim 3 comprising the nucleotide sequence shown in SEQ ID NOS. 3, 8, 11 or 14, or a fragment cDNA selectively hybridized to the cDNA.
6. A replication or expression vector carrying the cDNA according to claims 3 to 5.
7. A host cell transformed with the replication or expression vector according to claim 6.
8. A method for producing the polypeptide according to claim 1 or 2 which comprises culturing a host cell according to claim 7 under a condition effective to express the polypeptide according to claim 1 or 2.
9. A monoclonal or polyclonal antibody against the polypeptide according to claim 1 or 2.

10. A pharmaceutical composition containing the polypeptide according to claim 1 or 2 or the antibody according to claim 9, in association with pharmaceutically acceptable diluent and/or carrier.

Abstract

A new human polypeptide, a cDNA encoding the same and a pharmaceutical use of it.

The polypeptides of the present invention possess hematopoiesis regulating activity, tissue generation/regeneration activity, activin/inhibin activity, chemotactic/chemokinetic activity, hemostatic and thrombolytic activity, and receptor/ligand activity, therefore, they are expected to be useful for prevention and/or treatment of various diseases.

SEQUENCE LISTING

<110> Ono Pharmaceutical Co., Ltd.

<120> Novel Polypeptides, cDNA coding these polypeptides and Use thereof

<130> ONF-2975PCT

<141> 1999-05-13

<150> JP 10-131815

<151> 1998-05-14

<160> 19

<170> PatentIn Ver. 2.0

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Lys Trp Cys Leu Asp Pro Arg Val Val Leu Leu Ser Asn Thr Gln Thr

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Tyr Thr Cys Ser Val Gln Thr Asp Asn His Pro Lys Thr Ser Arg Val

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65 70 75 80
Pro Tyr Thr Cys Ser Val Gln Thr Asp Asn His Pro Lys Thr Ser Arg
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Val His Leu Ile Val Gln Val Ser Pro Lys Ile Val Glu Ile Ser Ser
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Thr Leu Gln Cys Glu Ala Ser Ala Val Pro Ser Ala Glu Phe Gln Trp

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Arg Lys Arg Tyr Leu Thr Ile Gly Leu Ser Ser Val Lys Arg Lys Lys
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Ile Phe Leu Leu Phe Met Asn Leu Tyr Ile Glu Asp Ser Tyr Val Leu	
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Ser Glu Arg Tyr Val His Thr Phe Lys Asp Leu Ser Asn Phe Ser Gly	
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Tyr Tyr Pro Ile Leu Asp Gly Leu Lys Arg Asn Tyr Asn Asp Pro Glu	
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Thr His Phe Arg Gly Leu Leu Ala Gln Lys Asn Val Ile Arg Phe Lys	
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Pro Asp Asn Pro Pro Ala Ser Leu Tyr Thr Asn Met Asn Val Phe Glu	
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aat tat gaa gca agc aag gct tac agt agt gtt gat gag tac ttt tgg	1220
Asn Tyr Glu Ala Ser Lys Ala Tyr Ser Ser Val Asp Glu Tyr Phe Trp	
355	365
ggg aaa cca cct tca aca gga gat gtt ttt gtg att gta ttt gaa aat	1268
Gly Lys Pro Pro Ser Thr Gly Asp Val Phe Val Ile Val Phe Glu Asn	
370	385
cca att ata ata aaa aaa att aaa gta aat act gga aca gaa gat cgg	1316

Pro Ile Ile Ile Lys Lys Ile Lys Val Asn Thr Gly Thr Glu Asp Arg

390

395

400

caa aat gat att ttg cat cat gga gcc cta gat gtt ggg gaa aac gtt 1364

Gln Asn Asp Ile Leu His His Gly Ala Leu Asp Val Gly Glu Asn Val

405

410

415

atg cct agc aaa caa agg gga caa tgt tct act tac tta aga cta gga 1412

Met Pro Ser Lys Gln Arg Gly Gln Cys Ser Thr Tyr Leu Arg Leu Gly

420

425

430

gaa ttc aaa aat gga aac ttt gaa atg tca ggt gta aat caa aaa att 1460

Glu Phe Lys Asn Gly Asn Phe Glu Met Ser Gly Val Asn Gln Lys Ile

435

440

445

cca ttt gat ata cat tgt atg agg ata tat gtc acc aaa aca caa aag 1508

Pro Phe Asp Ile His Cys Met Arg Ile Tyr Val Thr Lys Thr Gln Lys

450

455

460

465

gaa tgg cta att att agg agt att agc att tgg act tct tagccaatta 1557

Glu Trp Leu Ile Ile Arg Ser Ile Ser Ile Trp Thr Ser

470

475

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<211> 335

<212> PRT

<213> Homo sapiens

<400> 9

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				20						25					30
Ala	Leu	Ala	Tyr	Gly	Ser	Leu	Leu	Leu	Met	Ala	Leu	Leu	Pro	Ile	Phe
				35						40					45
Phe	Gly	Ala	Leu	Arg	Ser	Val	Arg	Cys	Ala	Arg	Gly	Lys	Asn	Ala	Ser
				50						55					60
Asp	Met	Pro	Glu	Thr	Ile	Thr	Ser	Arg	Asp	Ala	Ala	Arg	Phe	Pro	Ile
				65						70					75
Ile	Ala	Ser	Cys	Thr	Leu	Leu	Gly	Leu	Tyr	Leu	Phe	Phe	Lys	Ile	Phe
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Ser	Gln	Glu	Tyr	Ile	Asn	Leu	Leu	Leu	Ser	Met	Tyr	Phe	Phe	Val	Leu

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Gly Ile Leu Ala Leu Ser His Thr Ile Ser Pro Phe Met Asn Lys Phe			
115	120	125	
Phe Pro Ala Ser Phe Pro Asn Arg Gln Tyr Gln Leu Leu Phe Thr Gln			
130	135	140	
Gly Ser Gly Glu Asn Lys Glu Glu Ile Ile Asn Tyr Glu Phe Asp Thr			
145	150	155	160
Lys Asp Leu Val Cys Leu Gly Leu Ser Ser Ile Val Gly Val Trp Tyr			
165	170	175	
Leu Leu Arg Lys Val Phe Gly Thr Asn Val Met Val Thr Val Ala Lys			
180	185	190	
Ser Phe Glu Ala Pro Ile Lys Leu Val Phe Pro Gln Asp Leu Leu Glu			
195	200	205	
Lys Gly Leu Glu Ala Asn Asn Phe Ala Met Leu Gly Leu Gly Asp Val			
210	215	220	
Val Ile Pro Gly Ile Phe Ile Ala Leu Leu Leu Arg Phe Asp Ile Ser			
225	230	235	240
Leu Lys Lys Asn Thr His Thr Tyr Phe Tyr Thr Ser Phe Ala Ala Tyr			
245	250	255	
Ile Phe Gly Leu Gly Leu Thr Ile Phe Ile Met His Ile Phe Lys His			
260	265	270	
Ala Gln Pro Ala Leu Leu Tyr Leu Val Pro Ala Cys Ile Gly Phe Pro			
275	280	285	
Val Leu Val Ala Leu Ala Lys Gly Glu Val Thr Glu Met Phe Ser Tyr			

290	295	300	
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305	310	315	320
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<211> 1005

<212> DNA

<213> Homo sapiens

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<211> 1486

<212> DNA

<213> Homo sapiens

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<220>

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<222> (117)..(1121)

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Met

gac tcg gcc ctc agc gat ccg cat aac ggc agt gcc gag gca ggc ggc 167

Asp Ser Ala Leu Ser Asp Pro His Asn Gly Ser Ala Glu Ala Gly Gly

5

10

15

ccc acc aac agc act acg cgg ccg cct tcc acg ccc gag ggc atc gcg 215

Pro Thr Asn Ser Thr Thr Arg Pro Pro Ser Thr Pro Glu Gly Ile Ala

20

25

30

ctg gcc tac ggc agc ctc ctg ctc atg gcg ctg ctg ccc atc ttc ttc 263

Leu Ala Tyr Gly Ser Leu Leu Leu Met Ala Leu Leu Pro Ile Phe Phe

35

40

45

ggc gcc ctg cgc tcc gta cgc tgc gcc cgc ggc aag aat gct tca gac 311

Gly Ala Leu Arg Ser Val Arg Cys Ala Arg Gly Lys Asn Ala Ser Asp

50

55

60

65

atg cct gaa aca atc acc agc cgg gat gcc gcc cgc ttc ccc atc atc 359

Met Pro Glu Thr Ile Thr Ser Arg Asp Ala Ala Arg Phe Pro Ile Ile

70

75

80

gcc agc tgc aca ctc ttg ggg ctc tac ctc ttt ttc aaa ata ttc tcc 407

Ala Ser Cys Thr Leu Leu Gly Leu Tyr Leu Phe Phe Lys Ile Phe Ser

85

90

95

cag gag tac atc aac ctc ctg ctg tcc atg tat ttc ttc gtg ctg gga 455

Gln Glu Tyr Ile Asn Leu Leu Leu Ser Met Tyr Phe Phe Val Leu Gly

100

105

110

atc ctg gcc ctg tcc cac acc atc agc ccc ttc atg aat aag ttt ttt 503

Ile Leu Ala Leu Ser His Thr Ile Ser Pro Phe Met Asn Lys Phe Phe

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Pro Ala Ser Phe Pro Asn Arg Gln Tyr Gln Leu Leu Phe Thr Gln Gly			
130	135	140	145
tct ggg gaa aac aag gaa gag atc atc aat tat gaa ttt gac acc aag	599		
Ser Gly Glu Asn Lys Glu Glu Ile Ile Asn Tyr Glu Phe Asp Thr Lys			
150	155	160	
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Asp Leu Val Cys Leu Gly Leu Ser Ser Ile Val Gly Val Trp Tyr Leu			
165	170	175	
ctg agg aag gta ttt ggc acc aat gtg atg gtg aca gtg gcc aag tcc	695		
Leu Arg Lys Val Phe Gly Thr Asn Val Met Val Thr Val Ala Lys Ser			
180	185	190	
ttc gag gca cca ata aaa ttg gtg ttt ccc cag gat ctg ctg gag aaa	743		
Phe Glu Ala Pro Ile Lys Leu Val Phe Pro Gln Asp Leu Leu Glu Lys			
195	200	205	
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Gly Leu Glu Ala Asn Asn Phe Ala Met Leu Gly Leu Gly Asp Val Val			
210	215	220	225
att cca ggg atc ttc att gcc ttg ctg ctg cgc ttt gac atc agc ttg	839		
Ile Pro Gly Ile Phe Ile Ala Leu Leu Leu Arg Phe Asp Ile Ser Leu			
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Lys Lys Asn Thr His Thr Tyr Phe Tyr Thr Ser Phe Ala Ala Tyr Ile			

245	250	255	
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Phe Gly Leu Gly Leu Thr Ile Phe Ile Met His Ile Phe Lys His Ala			
260	265	270	
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Gln Pro Ala Leu Leu Tyr Leu Val Pro Ala Cys Ile Gly Phe Pro Val			
275	280	285	
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gag tca aat cct aag gat cca gcg gca gtg aca gaa tcc aaa gag gga	1079		
Glu Ser Asn Pro Lys Asp Pro Ala Ala Val Thr Glu Ser Lys Glu Gly			
310	315	320	
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Thr Glu Ala Ser Ala Ser Lys Gly Leu Glu Lys Lys Glu Lys			
325	330	335	
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<212> PRT

<213> Homo sapiens

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1 5 10 15

Asn Gly Asp Glu Ile Ser Lys Leu Ser Gln Leu Val Asn Ser Asn Asn

20 25 30

Leu Lys Leu Asn Phe Trp Lys Ser Pro Ser Ser Phe Asn Arg Pro Val

35 40 45

Asp Val Leu Val Pro Ser Val Ser Leu Gln Ala Phe Lys Ser Phe Leu

50 55 60

Arg Ser Gln Gly Leu Glu Tyr Ala Val Thr Ile Glu Asp Leu Gln Ala

65 70 75 80

Leu Leu Asp Asn Glu Asp Asp Glu Met Gln His Asn Glu Gly Gln Glu

85 90 95

Arg Ser Ser Asn Asn Phe Asn Tyr Gly Ala Tyr His Ser Leu Glu Ala

100 105 110

Ile Tyr His Glu Met Asp Asn Ile Ala Ala Asp Phe Pro Asp Leu Ala

115	120	125
Arg Arg Val Lys Ile Gly His Ser Phe Glu Asn Arg Pro Met Tyr Val		
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Leu Lys Phe Ser Thr Gly Lys Gly Val Arg Arg Pro Ala Val Trp Leu		
145	150	155
Asn Ala Gly Ile His Ser Arg Glu Trp Ile Ser Gln Ala Thr Ala Ile		160
165	170	175
Trp Thr Ala Arg Lys Ile Val Ser Asp Tyr Gln Arg Asp Pro Ala Ile		
180	185	190
Thr Ser Ile Leu Glu Lys Met Asp Ile Phe Leu Leu Pro Val Ala Asn		
195	200	205
Pro Asp Gly Tyr Val Tyr Thr Gln Thr Gln Asn Arg Leu Trp Arg Lys		
210	215	220
Thr Arg Ser Arg Asn Pro Gly Ser Ser Cys Ile Gly Ala Asp Pro Asn		
225	230	235
Arg Ser Trp Asn Ala Ser Phe Ala Gly Lys Gly Ala Ser Asp Asn Pro		240
245	250	255
Cys Ser Glu Val Tyr His Gly Pro His Ala Asn Ser Glu Val Glu Val		
260	265	270
Lys Ser Val Val Asp Phe Ile Gln Lys His Gly Asn Phe Lys Cys Phe		
275	280	285
Ile Asp Leu His Ser Tyr Ser Gln Leu Leu Met Tyr Pro Tyr Gly Tyr		
290	295	300
Ser Val Lys Lys Ala Pro Asp Ala Glu Glu Leu Asp Lys Val Ala Arg		

305	310	315	320
Leu Ala Ala Lys Ala Leu Ala Ser Val Ser Gly Thr Glu Tyr Gln Val			
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<211> 1080

<212> DNA

<213> Homo sapiens

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<222> (59)..(1090)

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Ser Ile Cys Gly Gln Glu Lys Phe Phe Gly Asp Gln Val Phe Arg Ile
      -1  1                5                10
aat gtc aga aat gga gac gag atc agc aaa ttg agt caa cta gtg aat 145
Asn Val Arg Asn Gly Asp Glu Ile Ser Lys Leu Ser Gln Leu Val Asn
      15                20                25
tca aac aac ttg aag ctc aat ttc tgg aaa tct ccc tcc tcc ttc aat 193
Ser Asn Asn Leu Lys Leu Asn Phe Trp Lys Ser Pro Ser Ser Phe Asn
      30                35                40                45
cgg cct gtg gat gtc ctg gtc cca tct gtc agt ctg cag gca ttt aaa 241
Arg Pro Val Asp Val Leu Val Pro Ser Val Ser Leu Gln Ala Phe Lys
            50                55                60
tcc ttc ctg aga tcc cag ggc tta gag tac gca gtg aca att gag gac 289
Ser Phe Leu Arg Ser Gln Gly Leu Glu Tyr Ala Val Thr Ile Glu Asp
            65                70                75
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ggg caa gaa cgg agc agt aat aac ttc aac tac ggg gct tac cat tcc	385
Gly Gln Glu Arg Ser Ser Asn Asn Phe Asn Tyr Gly Ala Tyr His Ser	
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Leu Glu Ala Ile Tyr His Glu Met Asp Asn Ile Ala Ala Asp Phe Pro	
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Met Tyr Val Leu Lys Phe Ser Thr Gly Lys Gly Val Arg Arg Pro Ala	
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Val Trp Leu Asn Ala Gly Ile His Ser Arg Glu Trp Ile Ser Gln Ala	
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Thr Ala Ile Trp Thr Ala Arg Lys Ile Val Ser Asp Tyr Gln Arg Asp	
175 180 185	
cca gct atc acc tcc atc ttg gag aaa atg gat att ttc ttg ttg cct	673
Pro Ala Ile Thr Ser Ile Leu Glu Lys Met Asp Ile Phe Leu Leu Pro	
190 195 200 205	

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Val Ala Asn Pro Asp Gly Tyr Val Tyr Thr Gln Thr Gln Asn Arg Leu

210

215

220

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Trp Arg Lys Thr Arg Ser Arg Asn Pro Gly Ser Ser Cys Ile Gly Ala

225

230

235

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240

245

250

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255

260

265

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270

275

280

285

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Lys Cys Phe Ile Asp Leu His Ser Tyr Ser Gln Leu Leu Met Tyr Pro

290

295

300

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305

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315

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Val Ala Arg Leu Ala Ala Lys Ala Leu Ala Ser Val Ser Gly Thr Glu

320

325

330

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Tyr Gln Val Gly Pro Thr Cys Thr Thr Val Leu

335

340

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<400> 19

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27

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL POLYPEPTIDES, cDNA ENCODING THE SAME, AND USE OF THEM

the specification of which is attached hereto unless the following box is checked:

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information of which is material to the patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

P. Hei. 10-131815

Japan

14/May/1998

(Number)

(Country)

(Day/Month/Year Filed)

☐

(Number)

(Country)

(Day/Month/Year Filed)

☐

(Number)

(Country)

(Day/Month/Year Filed)

☐

I hereby claim the benefits under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

I hereby claim the benefits under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

I hereby appoint John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon, Reg. No. 32,778; Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102; Brett S. Sylvester, Reg. No. 32,765 and Robert M. Masters, Reg. No. 35,603; my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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